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(54) Title: BIOACTIVE IMPLANTS

(57) Abstract: Bioactive electrodes for implantation into existing muscles are provided that enhance the functioning of the muscles. The electrodes may be coated with myogenic cells and may be transplanted as a living package, surrounded by a biologically degradable covering, into a weak muscle. Electrode surfaces may be coated with biologically active substances such as scaffold proteins that encourage migration and binding of myogenic cells and precursors to neurons. An electrode may slowly release trophic factors and may pre-train associated myogenic cells and/or nerve cell precursors by repeated electrical stimulation of the electrode in vitro in the presence of the cells. By transplanting myogenic cells along with the electrode, a new and powerful tool for muscle boosting, repair or augmentation is provided.

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Bioactive Implants

Field of the Invention

The invention relates generally to clinical improvement of body function by the use of implantable devices and biologics, and more specifically to coatings and other treatments of implants.

Background

A wide variety of applications require implantation of devices such as capsules, electrodes, and other solid surfaces into the body. Such applications are expected to increase as new technology becomes developed to address the neurological and other physiological problems associated with disease, injury and aging. Unfortunately, many materials used in these applications are not designed specifically for long term contact with internal body spaces and yield poor performance. Furthermore, many materials are designed to accommodate implantantion by for example, the use of hooks in electrodes to maintain their position during body movement. This field is in its infancy and much needs to be done to improve the performance of these devices.

Summary of the Invention

It was surprisingly discovered that electrodes, sensors and other devices for long term chronic stimulation of muscle(s) could be made more effective in several respects by adding bioactivity to them. The term "bioactive" in this context means that the electrode provides one or more biological activities in addition to merely conducting an electric pulse. Bioactivity is added by incorporating one or more proteins and/or cells to the surface(s) of the electrode and, in some cases, into porous spaces of the electrode. Each bioactive embodiment that arises from either protein activity, cellular activity or combinations of both, provides additional functioning to the electrode, that, in many cases emulates a naturally occurring process within the body.

One desirable cellular bioactive embodiment, for example is the use of diffusible cellular attractants associated with tissue damage and repair to solicit myoblasts to a desired site. Building this capability into the electrode, and/or

electrode covering allows the targeting of myoblasts that can not only cover and help hold the electrode in place, but can assist the transfer of electrical pulse excitation to the target muscle, while adding bulk to the muscle. Another cellular bioactive embodiment is the covering of at least part (eg, at least 10%, 25%, 50%, 75%, 85%, 90%, 95%, 98% or even all) of the electrode surface with one or more multinucleated living muscle myotubes, which assist incorporation and immobilization of the electrode into the underlying targeted muscle. Yet another cellular embodiment is the addition of one or more neurons, or neuron precursor cells into the electrode itself. The cells in this case may live within porous spaces of a conducting material of the electrode, or may form or participate in the conductor and be confined within a structural feature of the electrode such as within collagen or fibronectin sheets specifically polymerized in a form for this purpose.

A protein bioactive embodiment, for example, is the coating of the electrode surface with an extracellular matrix attachment protein. This coating facilitates binding to muscle cells, and particularly myoblasts, and can, in some embodiments both help immobilize and facilitate transmission of a nerve excitatory pulse. In another embodiment the electrode is coated with one or more substances such as antibody binding sites or other binders that can adhere to a desired substrate, such as the surface of a target muscle. In some embodiments such bindings can overcome the need to add a mechanical anchor such as a hook or multiple spikes that imbed the electrode in the target muscle. In another embodiment a coating protein both binds to integrin receptors on myoblast cells and stimulates the cells to fuse and thereby participate in the electrode-muscular junction. In another embodiment a coating on the electrode and/or cells attached to the electrode slowly release neurotrophic agents that facilitate neuronal growth to the electrode and improve sensitivity of activation. In yet another embodiment cells, such as myogenic cells are introduced with an electrode to enhance muscle function. A variety of further embodiments and their combinations readily will be appreciated upon a reading of the specification as further summarized below.

Description of the Preferred Embodiments

1. Bioactive Protein-Modified Electrodes

Bind Myoblasts and Myotubes with Extracellular Matrix Protein

In a desirable embodiment an electrode contains a surface of extracellular matrix protein that binds, preferably specifically to myoblast cells and/or mature muscle tube surface. A variety of myoblast scaffold proteins are contemplated that can be affixed to the electrode surface by a variety of techniques. These proteins include laminin, laminin-1, fibronectin, a collagen, type I collagen, type II collage, type IV collagen, thrombospondin-I, lecithin-oxytetracycline-collagen matrix, a galactin, galectin-1, vitronectin, and von Willebrand protein.

Attract Myoblasts with Migratory Factors

In advantageous embodiments myoblasts are prepared in culture and introduced into the body where they can fuse with existing muscle tissue. Most desirably, a target tissue is created by adding migratory attractants, in a preferably leachable form, which create a concentration gradient suitable for the myoblasts to follow. The attractants may be added to implanted electrodes, preferably porous electrodes, which may comprise a gel, hydrogel, complex surface, colloidal space, etc. that has leachable myoblast attractants, which slowly dissipate, creating a signal for myoblasts to follow. Myoblasts find the electrode surface and can fuse with muscle tissue there, preferably induced by one or more differentiation factors, and/or bind to scaffold proteins there such as fibronectin, and settle down.

The attractants can be added to a muscle surface by planting a substance, such as a patch, glue, gel or other material that stays at the muscle surface, but which slowly releases the attractants. Migratory myoblasts that are attracted to the muscle surface can fuse with and add bulk to the muscle. This is particularly useful in combination with implanted electrode(s) at the muscle surface, to help hold the electrode there by building up bulk near, over and around the electrode, as well as assisting in conductivity.

Migratory factors contemplated include, for example, crude extracts of injured muscle tissue, such as a water soluble low molecular weight extract from minced muscle that has been allowed to sit in culture media for 5 hours after mincing. This kind of extract can be prepared with a 30,000 molecular weight or 100,000 molecular weight cutoff filter. Within such kind of extract a number of proteins and other factors can be purified that can act as attractants. U.S. No. 6,284,242 issued to Kurachi on September 4, 2001 describes the use of basic fibroblast growth factor and fibronectin in this context. U.S. application No. 20010055590 (December 27, 2001) to this same

group further describes desirable factors such as cytokines that may be used in this embodiment. In particular, PDGF, HGF, fibronectin, MMP-1 and MMP-2 may be manipulated and used, as for example described for migration of myogenic precursor cells during development (Daston et al "Pax-3 is necessary for migration, not differentiation, of limb muscle precursors in the mouse" Development 122:1017-1027, 1996; Bladt et al. "Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud" Nature 376:768-771, 1995; Venkatsubramanian and Solursh "Chemotactic behavior of myoblasts" Devel Biol 104:406-407, 1984; Krenn et al. "Hyaluronic acid influences the migration of myoblasts within the avian embryo wing bud" Am J. Anat 192:400-406, 1991; Brand-Saberi et al. "Differences in fibronectin-dependence of migrating cell populations" J Embyol 187:17-26, 1993; Chin and Werb "Matrix metalloproteinases regulate morphogenesis, migration and remodeling of epithelium, tongue skeletal muscle and cartilage in the mandibular arch" Development 124:1519-1530, 1997).

Trophic Factors for Myoblast Survival, Replication and Maintenance of Myotubes

Trophic factors known to skilled artisans desirably are added to transplants, either directly or by transgenic expression from cells added to the implant. For example, a recently described family of growth factors, the neuregulins, are synthesized by motor neurons (Marchioni et al. Nature 362:313, 1993) and inflammatory cells (Tarakhovsky et al., Oncogene 6:2187-2196 (1991)). The neuregulins and related p185.sup.erbB2 binding factors have been purified, cloned and expressed (Benveniste et al., PNAS 82:3930-3934, 1985; Kimura et al., Nature 348:257-260, 1990; Davis and Stroobant, J. Cell. Biol. 110:1353-1360, 1990; Wen et al., Cell 69:559, 1992; Yarden and Ullrich, Ann. Rev. Biochem. 57:443, 1988; Holmes et al., Science 256:1205, 1992; Dobashi et al., Proc. Natl. Acad. Sci. 88:8582, 1991; Lupu et al., Proc. Natl. Acad. Sci. 89:2287, 1992). Recombinant neuregulins have been shown to be mitogenic for peripheral glia (Marchionni et al., Nature 362:313, 1993) and have been shown to influence the formation of the neuromuscular junction (Falls et al., Cell 72:801, 1993). Thus the regenerating neuron and the inflammatory cells associated with the recovery from neurogenic disease and nerve injury provide a source of factors which coordinate the remyelination of motor neurons and their ability to form the appropriate connection with their target. After muscle has been innervated by introduced neurons from an

implant, the motor neuron may provide factors to muscle, stimulating muscle growth and survival.

Other factors such as neuregulin can be used for increasing myotube formation or survival or muscle cell mitogenesis, differentiation or survival as taught for example by U.S. 6,444,642 issued to Sklar et al on September 3, 2002 and are useful for embodiements of the invention. For example, Fibroblast growth factor (FGF) is mitogenic for muscle cells and is an inhibitor of muscle differentiation. Transforming growth factor .beta. (TGF.beta.) has no effect on myoblast proliferation, but is an inhibitor of muscle differentiation. Insulin-like growth factors (IGFs) have been shown to stimulate both myoblast proliferation and differentiation in rodents. Platelet derived growth factor (PDGF) is also mitogenic for myoblasts and is a potent inhibitor of muscle cell differentiation and are used where myoblast growth is desired, but not differentiation or fusion.

Trophic Factors for Nerve Cell Growth and Maintenance

A variety of neurotrophic agents are known and used in embodiments of the invention. In each case, the amount needed can be determined by routine assay, but generally is between 0.01ng/ml to 100ng/ml. Other nerve cell factors useful for neurotrophic activity include a) IGF-I and IGF-II, which can be efficacious independent of any combination with insulin, b) combinations with protein kinase C activators, c) combinations with insulin, IGF-I and/or IGF-II, and various neurotrophic factors which can have additive effects on nerve cells. Of course, the involvement of glia and Schwann cells, which express insulin, IGFs and neurotrophic factor receptors further are useful in stimulating nerve cells. Furthermore, other neurotrophic factors such as the insulin-like growth factors (IGFs) and nerve growth factor (NGF) have activity and also are useful additives to implanted solid surfaces and as releasable materials according to embodiments of the invention.

A large set of neurotrophins may be used in embodiments of the invention. In fact, in several aspects, the survival and maintenance of differentiated function of vertebrate neurons is influenced by the availability of specific proteins referred to as neurotrophins. The neurotrophins form a highly homologous family of growth factors that are important for survival and maintenance of neurons during developmental and adult stages of the vertebrate nervous system (for review see Snider, 1994). Limited production of neurotrophins results in death of superfluous neurons (for

reviews, see Snider, W. D. & Johnson, E. M. (1989) Ann. Neurol., 26, 489-506, Barde, Y.-A. (1989) Neuron, 2, 1525-1534. The various neurotrophins differ functionally in their ability to support survival of distinct neuronal populations in the central and the peripheral nerve system Davies et al., J. Neuroscience, 6, 1897 (1986): Davies, A. M., Trends in Genetics, 4, 139-143 (1988), Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M. and Yancopoulos, G. D. (1990) Science 247, 1446-1451. The neurotrophin family is a highly homologous family which includes NT-3 Rosenthal A, Goeddel, D. V., Ngyuen, T., Lewis, M., Shih, A., Laramee, G. R., Nikolics, K., and Winslow, W. (1990) Neuron 4, 767-773, Hohn, A., Leibrock, J., Bailey, K., and Barde, Y.-A., Nature, 344, 339-341,1990, Kaisho Y, Yoshimura, K. and Nakahama, K. (1990) FEBS Lett. 266, 187-191, Ernfors, P., Ibanez, C. F., Ebendal, T., Olson, L., and Persson, H. (1990) Proc. Natl. Acad. Sci. USA 87, 5454-5458, Jones, K. R. and Reichhardt, L. F. (1990) Proc. Natl. Acad. Sci. USA, 87, 8060-8064, nerve growth factor (NGF) Levi-Montalcini, R. and Angeletti, P. U. (1968) Physiol. Rev., 48, 534-569, Thoenen H., Bandtlow, C. and Heumann, R. (1987), Rev. Physiol. Biochem. Pharmacol., 109, 145-178, brain-derived neurotrophic factor (BDNF) Barde, Y.-A., Edgar, D. and Thoenen, H. (1982) EMBO J., 1, 549-553, Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., and Barde, Y.-A. (1989) Nature, 341, 149-152 and neurotrophin 4/5 (NT-4/5) Hallsbook, F. et al., (1991) Neuron, 6, 845-858, Berkemeier, L. R., Winslow, J. W., Kaplan, D. R., Nikolics, K., Goeddel, D. V. and Rosenthal, A (1991) Neuron, 7, 857-866, Ip, N. Y., Ibanez, C. F., Nye, S. H., McClain, J., Jones, P. F., Gies, D. R., Belluscio, L., LeBeau, M. M., Espinsosa, R., III, Squinto, S. P., Persson, H. and Yancopoulos, G. D. (1992) Proc. Natl. Acad. Sci., 89, 3060-3064, and neurotrophin-6 (NT-6) (Barde, 1991; Gotz et al., 1994).

3F8 (phosphacan) and neurocan, another chondroitin sulfate proteoglycan of brain whose primary structure has recently been described (Rauch, U. et al., (1992) J. Biol. Chem. 267:19536-19547), interact with neurons and the neural cell adhesion molecules, Ng-CAM and N-CAM (Grumet, M. et al., (1993) J. Cell Biol. 120:815-824). The brain proteoglycans bind with high affinity (K.sub.d of approximately 0.5 nM) to Ng-CAM and N-CAM but not to other cell surface and extracellular matrix proteins such as laminin, fibronectin, several collagens, or receptors for epidermal growth factor (EGF) or fibroblast growth factor (FGF), or the myelin associated glycoprotein (Milev, P. et al., (1993) Glycobiology 3:535). A sequence of 1616 amino

acids deduced from a 4.8 kb open reading frame contains the N-terminal amino acid sequence of the 3F8 core glycoprotein as well as four internal CNBr, tryptic, and endo Lys-C peptide sequences from the proteoglycan.

When adding nerve cells to the compositions and devices described herein, a guide template may be placed over a desired region to direct nerve growth stimulated by neurotrophic factors. For example, the guide template may be a guiding filament (e.g., collagen, laminin or fascia) or a conduit (e.g., a wrapper, a cuff, or a tube), a porous electrode such as carbon, or a surgically prepared tunnel (e.g. laser, electrosurgical cautery, or auger mechanical tunnel). The conduit may be made of various materials derived from natural sources or synthetic materials. The conduit may be biodegradable or non-absorbable. Examples of conduit materials include but not limited to decalcified bone and vessels, fascia lata, fat, muscle, parchment, Cargile membrane, gelatin, agar, rubber, fibrin film, and various metals. The conduit desirably is filled with nerve-growth-stimulating agents such as nerve growth factors.

In a desirable embodiment one or more trophic factors are added to the interior or surface of the electrode, thereby inducing neurons to grow towards the electrode. In another desirable embodiment one or more protein trophic factors are made transgenically or by induction from a native gene of myocytes that are transplanted before, with or after implantation of the electrode.

Slow Release of Factors

These factors can be loosely bound by a variety of slow release technologies including for example, the salt composition complexes taught by Igari et al. (U.S. no. 6,376,461) and Johnson (U.S. No. 6,051,259 polymeric matrix of a biocompatible polymer and particles of biologically active, metal cation-stabilized hGH, wherein said particles are dispersed within the biocompatible polymer); hydrogels such as those made from poly(vinyl alcohol) (see U.S. No. 6,231,605); collagen, polyacrylamide, and the like. Most desirably the factors are included in a gel or resin material that can be implanted on the muscle surface by injection with a syringe to the desired area. In another embodiment the syringe injects the materials into the target muscle(s) itself, and slowly leaches out.

2. Bioactive Cell-Modified Electrodes and Piezoelectric Transducers

Coat Electrodes/piezoelectric devices with Muscle Prior to Implantation

In a desirable embodiment an electrode is coated with muscle tissue prior to implantation. Preferably the muscle tissue is immunologically compatible with the body being implanted. In a desirable embodiment myoblasts, which express tissue transplantation antigens are prepared and then contacted with electrode surface(s), which may be pretreated with one or more scaffold proteins such as fibronectin to facilitate myoblast binding. The myoblasts are induced to fuse, by for example adding chondroitin sulfate, or through another method. After covering the solid surface and fusing, the cellular material loses its MHC1 immunohistocompatibility antigens and can be implanted. In a related method, muscle cells that lack tissue transplantation antigens can be made available, for example, from transgenic porcine. More preferably the coating muscle tissue is genetically similar (taken by muscle cell culture from a relative) or identical, and obtained by culture of muscle cells sampled from the patient.

Sampling, preparation and culture of myoblasts has been carried out for many years due to the pioneering activities of Dr. Peter K. Law of Memphis. The extensive literature of the Law laboratory provides details for these procedures. For example, it was found that eliminating fibroblast overgrowth is very important to success in myoblast transplantation. Accordingly, in a desirable embodiment a "pure" culture of at least 90%, 95%, 97%, 98% or even above 99% myoblasts is prepared and the cells exposed to the solid surface (again, preferably pre-coated with a scaffold protein(s) such as fibronectin). After fusion, the solid part is implanted.

Although the cells produced are not as pure, other techniques may be used. For example, U.S. No. 5,833,978 issued to Tremblay on November 10, 1998 describes that myogenic cells may be any type of contractile cells including skeletal myoblasts including satellite cells, bone marrow stromal cells, peripheral blood stem cells, post natal marrow mesodermal progenitor cells, smooth muscle cells, adult cardiomyocytes, fetal cardiomyocytes, neonatal cardiomyocytes, embryonic stem cells, various cell lines, bone marrow derived angioblasts, endothelial cells, endothelial progenitor cells, or combinations thereof. The myogenic cells selected for coating solid surfaces should be able to differentiate into muscle cells preferably before or following exposure to the solid surface. Desirably, the cells are autologous to reduce chances of immune response and the possibility of introducing foreign proteins or viruses. Thus, in the case of autologous cell coatings used for the

implant, the myogenic cells are obtained from an individual, cultured and implanted back into the individual as coating(s) on solid surfaces. If myoblast cells from a non-autologous source are to be implanted, by themselves or in combination with fused muscle material, immmunosuppressants may be administered to the recipient.

Following isolation from a suitable source, the myogenic cells may be implanted fresh or may be expanded and/or purified in culture. Methods for expanding and purifying various cell types are well known to those skilled in the art. For example, details of such methods can be obtained from U.S. Pat. Nos. 5,130,141, 6,110,459, and 5,602,301. Myogenic cells obtained by sampling may be cultured in flasks in the presence of various growth factors including, but not limited to, vascular endothelial growth factors (VEGF), fibroblast growth factors (FGFs). In one procedure, typically, one day after starting culture, the culture medium of some flasks may be replaced by medium containing 100 ng/ml human recombinant bFGF (Sigma). Three days after starting culture, myoblasts are detached from the flasks with 0.1% trypsin followed by three suspensions in HBSS and centrifugations (6500 RPM, 5 minutes). The final cell pellet typically may be diluted in only 40 .mu.l of HBSS. Basic FGF may be used to both stimulate proliferation and inhibit differentiation of skeletal myoblasts in vitro. Other growth or trophic factors like insulin growth factor I, transferrin, platelet-derived growth factor, epidermal growth factor, adrenocorticotrophin and macrophage colony-stimulating factor as well as C kinase proteins activators or agonists by which the effect of bFGF is mediated may also have similar or even better effects than bFGF on the success of myoblast culture and use as a solid surface coating. Most desirably, an automated processor, such as that described by U.S. 6,261,832 issued July 17, 2001 to Law may be used.

In a desirable embodiment a solid body such as an electrode, piezoelectric transducer, implantable device or even wire leads implanted in the body are first coated with one or more scaffold proteins and then incubated with myoblasts. The myoblast concentration and trophic state may be adjusted for optimum spreading and covering of the solid part. Fusion may be started before contact with the solid part. Preferably the solid part is covered extensively, even until contact inhibition, and then fusion is induced. After complete fusion (e.g. after more than 75%, 85%, 90% or 95% or even more than 98% of the cells have fused) the coated part may be again exposed to myoblasts and another round of fusion incurred to increase the mass. This process may be repeated. One or more angiogenesis factors, which

may be added to the solid part, its surface, transgenically expressed by coated cells, or added to a surrounding shell may be used to stimulate vessel growth near the implant site. Angiogenesis factors and methods for their slow release (or slow synthesis) are known to the skilled artisan and not further detailed here.

In an embodiment the solid part is further encapsulated with a biodegradable shell such as collagen or other material, to allow insertion into the body without excessive mechanical trauma to the muscle coating. In another embodiment, multiple layers, or increasing thickness of the muscle coat is prepared by adding more myoblasts with optional myotubes and letting them all fuse in a mass. In yet another embodiment exterior bound myoblasts are added before transplantation but not allowed to completely fuse. In some instances the unfused myoblast layer(s) helps incorporation of the fused layer surrounding the solid part, into the body, by providing fusion with the desired body muscle, while at the same time fusing with the muscle layer of the solid part. Such "sandwich" of multinucleated body muscle-free myoblast-multinucleated solid part covering can be made to fuse, for example, by adding chemicals such as some high molecular weight chrondroitin sufates.

A wide variety of solid materials may be used, including stainless steel, polyethylene, and Teflon. In a desirable embodiment microstructured films and fibers of genetically engineered proteins are used to create films with controlled morphologies. For example, processing of biologically active polymers and polymer blends onto solid surfaces such as silicon micromachined substrates is known as exemplified by Patrick Tresco's group at the University of Utah and as funded by Protein Polymer Technologies, Inc. An electric field-mediated deposition process allows reliable and fast creation of thin fibrous coatings on solid substrates. Furthermore, electrochemical deposition processes allow deposit of blends of conducting polymers and bioactive proteins or peptides directly on the active electrode sites of microfabricated prosthetic devices as described by research proposals and publications of this group.

The coated solid devices may be implanted using regular surgical techniques. One concern in implantation is the presence of connective tissue sheaths surrounding both fascicles and individual myofibers. Myoblasts must first traverse these barriers to access the myofiber surface in order to fuse with and incorporate into the myofiber syncytium. Moreover, human muscle contains thicker connective tissue sheaths than that of smaller organisms, and therefore this barrier may be even

greater in humans than in experimental animal models such as mice. Thus, when using myoblasts and coated solids, it is desirable to open cross connective tissue barriers during implantation. In one embodiment the myofiber basal lamina is crossed. The lamina can be a barrier to viral-mediated in vivo gene transfer as Huard et al. "The basal lamina is a physical barrier to herpes simplex virus-mediated gene delivery to mature muscle fibers" J. Virol. 70:8117-8123, 1996).

In a desirable embodiment physical and chemical disruption of the basal lamina by damaging the muscle allows the myoblasts on or at the implant surface to cross the basal lamina and merge regenerating muscle fibers with a mosaic of endogenous and implanted myonuclei. Some embodiments have used either physical elimination to achieve this (Wernig et al. "Formation of new muscle fibers and tumors after injection of cultured myogenic cells" J. Neurocytol. 20:982-997, 1991; Morgan et al. "Normal myogenic cells from newborn mice restore normal histology to degenerating muscles of the mdx mouse" J. Cell Biol. 111:2437-2449, 1990) or myotoxic agents (Salminen et al. "Implantation of recombinant rat myocytes into adult skeletal muscle: a potential gene therapy" Human Gene Therapy 2:15-26, 1991; Bonham et al. "Prolonged expression of therapeutic levels of human granulocyte-stimulating factor in rats following gene transfer to skeletal muscle" Human Gene Therapy 7:1423-1429, 1996) to produce this effect.

Incorporate Passive electrodes, Nerve Cells, Glial Cells for Improved Muscle Stimulation

In many embodiments using present technology, an electric conductor is implanted to propagate electrical impulses from a signal generator. The signal generator may be implanted with its own power supply. For example, the Bion system (see for example, http://www.ami.usc.edu/Projects/Bion/docs/bionchfn.pdf and http//ami.usc.edu/Projects/Bion/docs/IFESS01_Bclin.pdf),has been developed the eventual aim of having these and further capabilities. Optionally the signal generator may be an exterior energy source, such as radio frequency energy that penetrates the skin, is picked up by part or all of the electrode/lead (generally by resonance) and converted into a voltage in the implanted electrode/lead. One or more diodes may be included as part of the implanted passive conductor, to convert the electromagnetic wave into a direct current pulse. For example a diode can be electrically connected in series with two portions of the conductor. Two diodes can

be used, optionally in parallel but in opposite polarity to generate bipolar signals. Two diodes or other electric components can be used to terminate and define a resonating structure that selectively absorbs energy of a specific wavelength intended for that structure.

One advantage of using anti-parallel diodes in this fashion is that a defined voltage level can be achieved through voltage clamping by each diode that conducts voltages over a given voltage level, determined by the structure of the diode. Multiple diode junctions and conductors/resistors can be added or removed (before implantation or in situ) to adjust the voltage that is created and or conducted by diode action. An electromagnetic energy emitter, such as a very high gigahertz antenna may be placed over the skin near the imbedded electrode and induces an electrical pulse by emitting energy that resonates with the imbedded system. While limited to the amount of energy imparted to a muscle system by the low efficiency transfer of radiowave to antenna, this system will become more important in the future as more effective electrode systems are developed that require less current to activate muscle cells.

In an advantageous embodiment one or more nerve cells are imbedded in the electrode and/or conductive lead, which may be porous. This embodiment allows regular electric circuit stimulation of an electrode through a conductive lead, but one or more neurons on or in the lead and/or conductive electrode can depolarize in response to an electrical pulse. The neuron can innervate or contact an electrically responsive membrane in the muscle and directly activate a muscle contraction. Such hybrid electrodes, that use regular electric conductivity to send pulses over a long distance, but use neurons at the distal tips to active muscle, can operate at lower electric energy levels compared to metal only electrodes that stimulate muscle directly without intervening neurons. Optionally, the electrodes include helper cells such as glial cells to assist the neurons. Accordingly, an embodiment includes a porous electrode such as conductive carbon fiber that contains neurons (and optionally glial cells). The electrode receives an electrical signal, which activates the neurons, which in turn activate muscle. The electrode, with its neurons attached, may be buried in muscle tissue.

Preferably the electrode is porous and contains nerve growth factors and even other factors that encourage the nerve cells to grow and establish contacts with the adjacent muscle tissue. In another embodiment the electrode includes not only

neurons with optional helper cells and growth factors, but also muscle cells such as myoblasts and even myotube coverings. Desirably the electrode has neural cell adhesion molecules on its surface. Ng-CAM is a large neuronal CAM of around 200 kDa that can mediate neuron-neuron and neuron-gila adhesion, and has been implicated in neuronal migration and the formation of nerve bundles. The biochemistry and biology of Ng-CAM is reviewed in Grumet, M., 1992, J. Neurosci. Res. 31:1-13, which is hereby incorporated by reference in its entirety. Ng-CAM binds homophilically (to itself) and heterophilically to several cell surface proteins. Ng-CAM is structurally related to human protein L1 (Reid, R. A. et al., 1992, J. Mol. Neurosci. 3:127-135), and binds to mammalian L1 (Grumet, M. et al., 1986, J. Cell Biol. 106:487-503; Lemmon, V. et al., 1989, Neuron 2:1597-1603). Desirably, purified Ng-CAM is used as a coating, as this protein when presented as a substrate for neurons in culture can promote neuritic fiber extension of about 100 .mu.m in several hours. This and other CAMs are useful for embodiments.

In fact, the binding of certain CAMs including L1 to neurons generates signals such as an increase in intracellular calcium that have been associated with promotion of neurite growth (Schuch et al., supra; Williams, E. J. et al., 1992, J. Cell Biol. 119:883-892). This and any other molecules which binds to and inhibit or enhance their function may have a significant impact on axonal growth to improve transmission of signals from the electrode to nerve cells and from nerve cells to muscle. Ng-CAM and N-CAM's ability in this context has been shown (see Daniloff, J. K. et al., 1986b, J. Cell. Biol. 103:929-945)). Various satellite cell growth factors are known and desirably used, particularly as diffusible agents, in embodiments of the invention. See, for example, U.S. No. 5,435,999 issued to Austin on July 25, 1995, which describes a useful leukemia inhibitory factor that stimulates satellite cells.

Molecules that inhibit binding of nerve cells are desired for coating the respective portions of electrodes/leads/transplanted surfaces where nerve cells are undesired. For example, the molecules phosphacan, a chondroitin sulfate proteoglycan, and 3F8, another proteoglycan as described in U.S. 5,625,040 issued to Margolis on April 29, 1997 may be used in combination to help order where nerve cells should not bind. For example, phosphacan can be used to coat insulating portions of an electrode, or other parts where electric triggering of nerve cell action potentials are to be avoided. In an embodiment, the active portion of an electrode is

coated with one or more neural cell adhesion molecules and the insulated portion is coated with phosphacan. Most desirably, a conductive electrode (optionally porous) is in contact with nerve cells only at its end. A nerve cell promoter such as Ng-CAM coats that end, and an inhibitor such as phosphacan coats adjacent areas of the electrode to keep the nerve cells from spreading into longer conductive lead regions of the electrode/lead systems where regular electric conductivity is relied on for transmission of a pulse. In this way, nerve cells are confined to the end region of an electrode where the muscle connection is made.

In practical use, coated electrodes may be incubated with nerve cells prior to implantation, or the nerve cells are added during or after implantation and find the desired surface of the electrode later. Most desirably glial cells may be added. For example, coated electrode ends may be incubated with glial cells to allow attachment, and then incubated with nerve cells. Finally the electrode is implanted. By using a porous electrode, and/or a overwrap coating such as collagen, the adhered cells are protected from mechanical dislodgement during implantation. The overwrap preferably is biodegradable and has a thickness that is determined by routine optimization, using an animal model system, with samples of different degrees of crosslinking and thicknesses. In an embodiment a smooth electrode is made fuzzy by adding a coating such as a hydrogel or large chondroitin sulfate chains that may be prepared by crosslinking. The fuzzy surface improves glial cell and nerve cell adhesion. Crosslinking is a very mature art. Glutaraldehyde, or more specific reagents may be used. U.S. No. 5,610,148 describes useful porous cell adhesion protein materials useful for embodiments. Also, see in particular the useful proteins and complexes described in Clark, R. A. F, "Fibronectin matrix deposition and fibronectin . . . ", J. Invest. Dermatol., 94/6 Suppl., pp. 128S-134S, 1990. Grinnell, F et al., "Distribution of Fibronectin during wound . . . ", J. Invest. Dermatol., vol. 76(3), 1981, pp. 181-189. Baur, Paul et al., "The myofibroblast anchoring strand . . . ", J. Trauma, vol. 23(10), pp. 853-862, 1983. Caffesse, R. G. et al., J. Clin. Periodontol., vol. 12(7), pp. 578-590, 1985. Chiquet et al: "Muschle morphogenesis: Evidence for anorganizing function of exogenous fibronectin"; Developmental Biology, vol. 88, No. 2, Dec. 1981, pp. 220-235 (especially p. 230--col. 2--p. 234). Simmons, "Evaluation of collagen cross-linking techniques for the stabilization of tissue matrices", Biotechnol-Appl-Biochem., 1993 Feb.; 17 (Pt 1):23-9. Freed, "Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46

months after transplantation for Parkinson's disease", N. Engl. J. Med., 1992 Nov. 26; 327(22):1549-55. Gershon, "Compliance and ultimate strength of composite arterial prostheses", Biomaterials, 1992; 13(1):38-43. Ricci, "In-vitro tendon cell growth on synthetic fiber implant materials: biological implications", Bull. Hosp. Jt. Dis. Orthop. Inst., 1990 Fall; 50(2):126-38. Matsuda, "Development of a novel artificial matrix with cell adhesion peptides for cell culture and artificial and hybrid organs", ASAIO. Trans. 1989 Jul.-Sep.; 35(3):677-9. Jakobson, "A simple method for shell-less cultivation of chick embryos", Pharmacol-Toxicol. 1989 Feb.; 64(2):193-5. Brown, "Therapeutic Uses of Cell-Matrix Adhesive Proteins", Current Opinion in Therapeutic Patents, Aug. 1993, pp. 1117-1140, which are useful for embodiments of the invention.

3. Coating of implantable materials with muscle tissue

Allogeneic and autologous myoblasts are particularly useful for coating biological materials, for example a pig heart valve, human damaged tissue, and non-biologics such as stents, plastic valves, plastic heart valves, other vessels, either biologic or synthetic, and any other implantable materials. A biological tissue in this context may be for example, a sphincter muscle, a skin, a tongue, an eyelid, an eardrum, stomach tissue, bladder tissue, vascular tissue, artery section, vein section, aorta section, heart tissue. A skilled artisan can optimize the amount and conditions for coating.

For example, a pig heart valve may be coated with myoblasts obtained from a muscle biopsy derived from the patient. Desirably, the pig heart valve, or other implant is first coated with a scaffold material such as fibronectin, laminnin and/or collagen prior to coating with myoblast cell(s). Desirably, the coating is thick enough to block sites of the uncoated surface from reacting with one or more components of the immune surveillance system. Crosslinking adsorbed material may be carried out by any of known conventionally means to both ensure long term attachment and a suitable thickness.

4. Implantation of Piezoelectric Feedback Devices and Systems

For many applications, electrode stimulation of a muscle is monitored to generate a feedback signal for tuning of the system. Such tuning may be used to determine optimum electrode signal, adjust for vagaries of electrode placement,

coordinate synergy among multiple muscles working together, to re-calibrate of electrode systems that decay in performance or otherwise altered their performance, and so on. Desirably the feedback is obtained from within the body and preferably from a muscle itself, such as by monitoring electric signals picked up from an electrode in the stimulated muscle. In a very desirable embodiment the physical motion of the muscle is monitored by imbedding a piezoelectric device in the muscle, preferably with a long axis parallel to the muscle fibers, or by acoustically coupling the piezoelectric crystal to the muscle, either by direct contact, or near contact with the surface.

A variety of piezoelectric materials may be imbedded within the body to generate piezoelectric pulses that correspond to muscle movement. The pulses can be amplified, filtered and input into a circuit that can use the pulses to monitor the effect of electrode(s) stimulation of muscle. Most preferably, a polymer such as a polymer of vinylidenfluoride and trifluoroethylene copolymer is used, with electrical insulation as suited and electrodes connected to the polymer to conduct the induced pulses to a circuit.

In a particularly desirable embodiment the imbedded piezoelectric sensor is rod shaped with crystalline or quasi-crystalline orientation selected to generate maximum signal upon bending of the long axis. The electrodes that conduct the piezoelectric signals should be flexible and expandable, such as a coiled spring, looped thread, and so on.

A circuit that receives the piezoelectric signal optionally has a high impedance front end buffer that converts the signal into a lower impedance (higher current) form, for analysis and comparison with desired and/or reference signals. For example, the circuit may compare the peak height of a detected signal with, for example, a sample and hold amplifier. The circuit may monitor the average signal strength of the signal. The circuit may monitor the total signal strength, for example by integrating the signal with a capacitor. The circuit may monitor the frequency and/or repeatedness of the piezoelectric generated signals. Such analyses may be carried out by computer analysis, using a stored program in a circuit that stores incoming data and calculates one or more properties. In one such embodiment the circuit and/or computer compares one or more properties of the incoming signal with a stored reference to determine whether the muscle stimulator is changing effectiveness and needs to have the pulse modified, or needs to alert the operator that a problem has

occurred.

5. Implantation of Myogenic Cells, Particularly Autologous Cells for Enhanced Muscle Function

Many patients that need electrode stimulation enhancement of muscle function have weakened muscles. In an embodiment of the invention, these muscles acquire improved function by transplanting myogenic cells before, during or after electrode implantation. Myogenic cells, such as autologous myogenic cells prepared from the patient beforehand, can be reintroduced at high levels, such as 106 to 107 cells, 107 to 108 cell, 108 to 109 cells, or even higher. The cells, once introduced, fuse with preexisting muscle cells, thus contributing their DNA and fortifying the bulk of the native muscle.

Synergy results from including myogenic cells with an electrode. The myogenic cells, when already attached to an electrode shorten the time required for the body to accommodate the electrode. In an embodiment, a smaller excitation current is required because of the accommodation of electrode with muscle cell in vitro, particularly when the muscle cell morphology is altered prior to implantation by the use of entraining currents in vitro. For example, cells that coat the electrode and other cells in the vicinity, in an embodiment, are electrically stimulated by the electrode during growth in culture. The stimulation may occur over a period of at least 1 hour, 3 hours, 6 hours, or 12 hours but preferably occurs for at least one day, two days or three days. This stimulation may facilitate the induction of genes involved with formation of the motor end plate and/or other features that desirably interact with the electrode. By increasing the amount of these features, a more sensitive electrode response may be had after implantation. In another desirable embodiment, nerve cell progenitors likewise are incubated with the electrode in vitro before implantation. In a desirable embodiment, nerve cells precursors and myogenic cells are incubated with the electrode in vitro and experience electrical activation of that electrode in vitro, which induces the cells to form structures that respond to the electrical activation. The amount of electrical current and voltage used as well as periodicity and wave form, preferably should be similar to that used on the electrode after implantation. Preferably the stimulation is biphasic, to alleviate effects of electrolysis, and the instantaneous current is less than 10 ma, more preferably less than 3 ma, and in some situations where repeated stimulations (more

than once per 5 minutes) occur over an extended time, less than 0.5 ma or even less than 100 microamperes.

Introduction of myogenic cells such as autologous cells that are fusion capable, can be made by direct injection, as pioneered by the work of Peter Law (see U.S. Nos. 5,130,141 and 6,261,832). In one embodiment, muscles of the patient are stimulated by needle probings, to facilitate generation of muscle satellite cells. Afterwards, one or more biopsies are performed, and a relatively pure culture of myocytes obtained. The myocytes may be directly injected into muscles that need improved function, including any and all muscles that are candidates for receiving electrode implantations. The myocytes may be, for example, injected 1, 2, 3, 4, 5, 7, 10, 14, 20 or more days prior to electrode implantation. Additionally, or instead, the myocytes may be added to one or more muscles in need of treatment with the electrode(s) destined for a particular muscle. In a particularly advantageous embodiment, myocytes and/or myotubes are transplanted attached or partially attached to the electrode. For example, about 1%, 2%, 5%, 10%, 25%,50%, 75%, 85%, 90% or more of the myocytes (by cellular mass) may be attached to the electrode as describe herein. In yet another embodiment, nerve cell stem cells are attached to the electrode or added with the electrode to the muscle.

To prevent mechanical tearing of cell-electrode assemblies, preferably an entire package of electrode with attached muscle (and or nerve cells or nerve cell precursors) is literally packaged within a protective sheath of material such as gelatin, or other polymer as is known in the surgery art. The polymer(s) used to cover the electrode with attached muscle should degrade into biologically acceptable end products after residence in the body.

Preferably autologous cells, obtained from the same patient are used, and obtained by tissue biopsy, as described for example in U.S. NOs. 6,099,832, 5,833,978, 6,284,242 and 5,130,141. In preferred embodiments, a composition is prepared from a myogenic cell or precursor to myogenic cell having a minimum contamination by fibroblasts. The term "minimum contamination by fibroblasts means that on a total cell number basis, less than 5%, 2%, 1% 0.5% 0.2% or even less than 0.1% of the cells are fibroblast cells. This improved composition purity can be achieve a variety of ways. For example, in one way, fibroblast cells are preferentially inhibited or killed by the inclusion of one or more substances in the culture medium.

In another way, a cell sorter is used that separate cells one at a time. In yet another way, a non-fibroblast specific promotor such as a muscle specific promoter is used to control expression of a gene that generates a product which allows a cell that makes that product survive in cell culture. In this way, transformed myogenic cells preferentially survive and the percentage of fibroblasts diminishes. In yet another way, the myogenic cells are cultured in the presence of a macrophage cytokine such as that described by Giurisato et al. in Basic Appl. Myol. 8(5): 381-388 (1998), which stimulates proliferation of myogenic cells but not fibroblast cells. The cytokine may be produced by culturing macrophages in a serum free medium and then harvesting the medium to obtain a crude preparation of cytokine. In a preferred embodiment basic cell transfer therapy techniques that utilize very purified (low fibroblast contamination) cultures are made possible by adding crude or partially purified preparations of a 50-10KDa cytokine secreted by macrophages to the culture and growing at least 2, 3, 5, 8 or 10 generations or more of the myogenic cells.

In each case some cell division may be used to increase cell number prior to use of the cells. Most desirably at least 1, 2, 5, 10, 25, 50 or even more than 100 billion cells are cultured and transferred. Where the cells are not autologous, it is generally preferred to utilize an agent to minimize or eliminate xenograft rejection such as cyclosporine, in the animal or patient that receives the cells. Further agents may be added to the reintroduced cells such as viscosity adjusting materials, adhesive agents and the like, to assist placement and positioning of the cells within (or even on) the muscle during or after cell transfer.

Myogenic cells, or precursors to myogenic cells are activitated or transformed to express one or more genes that stimulate endothelial cell growth and/or development of blood vessels. According to an embodiment of the invention the cells before transfer into a target muscle are transformed with one or more genes under the control of a suitable promotor that expresses an endothelial cell growth and/or angiogenesis protein. For example, both acidic and basic fibroblast growth factor molecules are mitogens for endothelial cells and other cell types and desirably are stably incorporated into cells that are or become myogenic cells. Angiotropin and angiogenin can induce angiogenesis, as described by Folknan, J., Cancer Medicine, Lea and Febiger Press, pp. 153-170 (1993). A highly selective mitogen for vascular endothelial cells is vascular endothelial growth factor or VEGF (Ferrara, N.

et al., Endocr. Rev. 13:19-32 (1992)), which is also known as vascular permeability factor (VPF). In most preferred embodiments VEGF is transgenically expressed. However, in some embodiments the desired gene is turned on by homologous recombination. Most preferably VEGF is transgenically expressed. In an embodiment at least two different genes are transgenically expressed such as VEGF with angiotropin or angiogenin. In another emobodiment more than two different genes are transgenically expressed. Multiple genes can be expressed within the same cell, or may be expressed by different cells within the same composition. In some circumstances expression of two different factors, such as two different angiogenesis factors synergistically results in greater establishment of the transplanted cells within a target diseased heart muscle.

Transformation of cells can be achieved by a variety of methods as will be appreciated by a skilled artisan. Generally a nucleic acid sequence encoding a desired polypeptide such as the VEGF165 gene is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the .beta.-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

In one embodiment a retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, .psi.-2, .psi.-AM, PA12, T19-14X, VT-19-17-H2, .psi.CRE, .psi.CRIP, GP+E-86, GP+envAml12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO.sub.4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. In this

embodiment the producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce myogenic cells or precursors to myogenic cells. The transduced cells express the nucleic acid sequence(s) encoding the polypeptide.

In a preferred embodiment cells are transformed with VEGF. VEGF has four different forms of 121, 165, 189 and 206 amino acids due to alternative splicing as described, for example in U.S. No. 6,040,157. VEGF121 and VEGF165 are soluble and are capable of promoting angiogenesis, whereas VEGF189 and VEGF206 are bound to heparin containing proteoglycans in the cell surface. The temporal and spatial expression of VEGF has been correlated with physiological proliferation of blood vessels (Gajdusek, C. M., and Carbon, S. J., Cell Physiol 139:570-579 (1989); McNeil, P. L., et al., J Cell. Biol. 109:811-822 (1989)). Its high affinity binding sites are localized only on endothelial cells in tissue sections (Jakeman, L. B., et aL., Clin. Invest. 89:244-253 (1989)). In an embodiment of the invention at least two types of cells are transplanted into differing regions of the muscle. One type of cell expresses VEGF (and optionally another angiogenesis factor) and preferentially is transplanted into region(s) where blood vessel growth is most desired. A second type of cell is transplanted into region(s) where blood vessel growth is less needed. A medical specialist can readily determine optimized locations for transplanting the two (or more) types of cells.

Vascular permeability factor (VPF) has also been found responsible for persistent microvascular hyperpermeability to plasma proteins even after the cessation of injury, which is a characteristic feature of normal wound healing. This suggests that VPF is an important factor in muscle wound healing. Brown, L. F. et al., J. Exp. Med.176:1375-1379 (1992). VEGF expression is high in vascularized tissues, (e.g., lung, heart, placenta and solid tumors) and correlates with angiogenesis both temporally and spatially. VEGF also has been shown to induce angiogenesis in vivo. Since angiogenesis is essential for the repair of normal tissues, especially vascular tissues, VEGF has been proposed for use in promoting vascular tissue repair (e.g., in atherosclerosis).

U.S. Pat. No. 5,073,492, issued Dec. 17, 1991 to Chen et al., discloses a method for synergistically enhancing endothelial cell growth in an appropriate environment, which comprises adding to the environment, VEGF, effectors and

serum-derived factor. Also, vascular endothelial cell growth factor C sub-unit DNA has been prepared by polymerase chain reaction techniques. The DNA encodes a protein that may exist as either a heterodimer or homodimer. The protein is a mammalian vascular endothelial cell mitogen and, as such, is useful for the promotion of vascular development and repair, as disclosed in European Patent Application No. 92302750.2, published Sep. 30, 1992. In embodiments of the invention co-expression of VEGF165 with VPF and/or vascular endothelial cell growth factor C is used, in the same cells or in the same composition of cells, for desirable synergistic effects.

6. Electrode Materials Suitable for Embodiments of the Invention

A variety of electrodes are contemplated for embodiments of the invention. Most technology developed thus far in this area uses simple metal electrodes such as stainless steel that generally have a coiled structure to allow extension for accommodating muscle contraction and relaxation. Generally, noble metals such as gold, iridium, platinum, rhodium, ruthenium, palladium, and silver, and passive metals such as copper, tin, indium, gallium tantalum, niobium, chromium aluminum titanium and tungsten may be used. However, organic conductors such as polypyrroles and carbon also may be used.

Most preferred is carbon that may be generated from a polymer such as a poly(p-phenylene vinylene), a polyacetylene, a poly(p-phenylene), a poly(p-phenylene sulfide), a polyanaline, a polyacetylene, polyacrylonitrile, a polyquinoline, a polypyrrole, and/or a polythiophene. Such carbon polymer based materials may be synthesized by an oxidizing reaction that converts one or more starting polymer(s) into a higher carbon content substance. This embodiment of the invention provides great control of the shape and size of the electrode and/or lead, which can be long, highly branched, mesh like etc depending on the structure of the polymer before oxidation. Thus, the polymer(s) can be formed into a strand, a mesh, a dendritic looking branched structure and so on followed by oxidizing the structure to increase the carbon context. Preferably the structure is oxidized by heating; and shaping the treated structure into a suitable two or three dimensional form. As described in the scientific literature, the material may be processed by heating in the presence of 10 percent or more oxygen to oxidize the polymer. After oxidation, the material is heated again in the absence of oxygen (i.e. less than 1%, 0.5%, 0.2%

0.1%, 0.02 %) and preferably as little oxygen as possible. Most desirably, the treated fiber has virtually 100% carbon content and exhibits an electrical resistance of 0.2 to 50 and more preferably 2-5 ohms per square meter across the width and 0.1 to 25 or more preferably 1 to 3 ohms per square meter along the length. Portions can be insulated or laminated using any available insulator. For example, U.S. No. 6,172,344 shows a range of suitable materials (see column 9 and table 1).

Early work with these kinds of polymers used polyacetylene, which was said to exhibit high electric conductivity upon oxidation. Later, such conjugated polymers were found to embrace other conjugated hydrocarbon and aromatic heterocyclic polymers, such as for example, poly(p-phenylene), poly(p-phenylene vinylene), poly(p-phenylene sulfide), polyacetylene, polyanaline, polyquinoline, polypyrrole, and polythiophene. See U.S. No. 6,172,344 issued to Gordon et al., which shows how to make a conductor fabric. Desirably, such fabric is shaped to assume the desired excitatory contours of a muscle and is electrically connected to a lead that may be of the same material or different. Also, the shaped fabric may be used as two separate pieces which may be connected to two leads or one lead plus a ground for bi-axial stimulation. In one embodiment the pieces are symmetrically shaped to allow generally equal area contact on two locations of the muscle. Also see U.S. No. 6,208,075 which describes the use of fluorocarbon polymers. Other techniques are included in this embodiment, such as electrochemical oxidation of resonance-stabilized aromatic molecules, structure modification, doping, and generation of transition metal-containing polymers. Each of these may be used along with metallic conducting materials for embodiments of the invention, not only for electrodes but for conducting leads as well.

Electrodes and can be coated with scaffold proteins such as fibronectin, portions of fibronectin and other substances as described herein. The coatings preferably are attached by covalent binding, although non-specific binding may be used. A wide variety of attachments are known. In one embodiment attachment is carried out by non-specific binding, which can be very firm, depending on the type of surface and the type of molecule, as shown for example by U.S. No. 5,043,288 issued to Motsenbocker on August 27, 1991. Most preferably the attachment is covalent. See for example, gas discharge methods reviewed in U.S. No. 5,055,316 issued to Hoffman et al. on October 8, 1991 and U.S. Nos. 6,379,741; 6,329,024;

6,090,995; 5,798,261; 5,591,140; 5,451,428; 5,437,900; 5,378,146; 5,342,693; 5,326,584; 5,260,093; 5,147,678; and 5,055,316. .

In a desirable embodiment the electrode is fuzzy, for increased surface area. The coating may be protein polymer, polymer conjugated to adhesion factor or adhesion factor fragment that binds to muscle and/or nerve or glial cells or a conducting polymer/biomolecule blend. For example, a conducting polymer such as a doped polypyrrole may be combined with a synthetic protein polymer having a fibronectin fragment including RGD (SLPF) and nonapeptide CDPGYIGSR. Any of the polymers described in the references cited herein may be used and further treated by coupling such peptide fragments or even whole scaffold proteins useful for binding glial cells, nerve cells and/or muscle cells. The coated material preferably is incubated with glial and neural cells that bind to and grow on the coating. Also preferably, this step is followed by incubation with myoblasts. In an embodiment the nerve cell incubated electrode is freed of exogenous nerve cells and then the surface coated, incubated or complexed with scaffold protein specific for binding muscle prior to the myoblast incubation step, to help order a structure with an inner electric conducting core (carbon or other organic conductor and/or metal) followed by glial/nerve cell layer, followed by muscle cell/tissue layer. This complex sandwich may be implanted directly or after coating with a biodegradable or dispersible capsule. Of course a porous structure in some embodiments will not have defined layers, but preferably has glial cells and neurons more deeply within the pores and muscle cell/tissue generally on the outside.

Although not described in detail herein, embodiments of the invention include coating and preparation of other implants such as artery and vein valves, heart valves, prostheses and other implantable materials. Generally, such implantables are coated with scaffold with optional diffusible migratory factors and myoblasts added before, during or after implantation.

A typical electrode size is 100 microns wide and 15 microns deep. Other electrodes may be up to 2 times, 5 times, 10 times, 100 times or even 1000 times as thick. The electrode may be buried into the muscle by virtue of having a hook configuration or other configuration as is generally known in the art. Most preferably the electrode (and/or lead) is fibrous and made from organic conductor such as carbon, with insulation where desired. A thin fiber of carbon, preferably with bioactive protein(s) and bioactive cells as described herein can be imbedded or

placed onto a muscle. After a period of time such as 12 hours, one day, two days, or a week, the bioactive conducting fiber is mechanically stabilized enough for continuous use. Most desirably the electrode has a myotube covering that fuses, with the target muscle.

In an embodiment myoblasts are added that fuse both with the electrode covering and the target muscle, thus bringing them together in multinucleated structures that contain an imbedded conducting fiber with optional nerve cell(s). The electrode (either with scaffold protein or without it) can be coated with myoblast cells, myotubes, or other fused myoblast muscle structure. Preferably this is carried out by incubating the electrode in vitro with myoblasts under conditions where myoblasts are ready to fuse. Desirably, a pre-coating treatment with fibronectin (or other scaffold protein) is used to initiate myoblast attachment such that subsequent fusion leads to muscle tissue that covers the electrode.

Of course, other mechanical and biological attachment mechanism made be used to affix electrodes to muscle. Molecular scale attachment mechanisms are used, in some embodiments of the invention for immobilization. In some cases, the muscle surface is first treated with one component of a multi component system, such as may be used for molecular Velcro. For example, the peptide intermolecular self-assembly hook systems explored by Dr. Shuguang Zhang at the Center for Biomedical Engineering may be adapted for this purpose. This technology may use self-assembling nanoscale materials based on chemical complementarity and structural compatibility. Several distinctive types of peptides in that system are particularly useful. Type I peptides undergo intermolecular self-assembly to form a gel matrix that can be used for slow release of imbedded molecules as well as for attachment to muscle and formation of electrodes. Type II peptides undergo structural transformations for intermolecular and intramolecular self-assembly. Type III peptides undergo self-assembly on to surfaces to form molecular hook and molecular Velcro that interact with other molecules and to control cell patterns. The self-assembling peptide systems are simple, versatile and easy to produce. Other adhesives, including so-called "biological glue" may be used as well.

Each publication cited herein is specifically incorporated by reference in its entirety.

I claim;

1. A method of focusing myoblast cells introduced into a patient to a desired muscle site, comprising:
providing a reservoir of one or more leachable substances that affect myoblast migratory behavior;
placing the reservoir in direct contact with the muscle in vivo to allow gradual release of the leachable substance.

2. The method of claim 1, wherein the leachable substance that affects myoblast migratory behavior is selected from the group consisting of platelet derived growth factor culture media extract from culture of macrophages, and low molecular weight culture media extract from culture of damaged muscle.

3. The method of claim 1, wherein the reservoir comprises a matrix material selected from the group consisting of a colloid, a gel, a fabric, a solid foam, cross linked protein, collagen, oriented fibronectin fibrils, a synthetic polymer, body tissue from another organism, body tissue from another individual, body tissue from another species, a metal, stainless steel, titanium, high molecular weight polyethylene, alloy, alloy of aluminum, alloy of tinanium..

4. The method of claim 1, wherein the reservoir is a thin patch that adheres to the muscle surface through specific binding selected from the group consisting of muscle antigen binding to immobilized antibody binding sites in the patch, muscle cell integrin binding to immobilized extracellular matrix protein sites in the patch.

5. The method of claim 3, wherein the body tissue is selected from the group consisting of a valve, a sphincter muscle, a skin, a tongue, an eyelid, an eardrum, stomach tissue, bladder tissue, vascular tissue, artery section, vein section, aorta section, and heart tissue.

6. The method of claim 3, wherein the body tissue is a heart valve.

7. The method of claim 3, wherein the metal is a surface of a stent, a catheter, an artificial joint, or a tooth.

8. A biological stent comprising a stent coated with at least one of myoblasts and a myoblast derivative selected from the group consisting of myotubes, muscle fibers, and fibromyoblasts.

9. A method of targeting myogenic cells introduced into a body for augmentation of an electrode operated muscle, comprising
placing an electrode, comprising a leachable substance in a matrix, that affects myoblast migratory behavior, into the muscle,
wherein the leachable substance slowly diffuses out of the electrode to create a concentration gradient suitable for attracting myoblasts to the electrode location.

10. The method of claim 9, wherein the electrode comprises a porous substance that has the leachable substance loosely associated with it.

11. A bioactive implant, comprising:
a solid implantable material with a cellular coating, the cellular coating comprising at least one of myoblasts, transduced myoblasts, transformed myoblasts, myotubes, muscle fibers, and fibromyoblasts.

12. The bioactive implant of claim 11, wherein the solid implantable material comprise at least one of a colloid, a gel, a fabric, a solid foam, cross linked protein, collagen, oriented fibronectin fibrils, a synthetic polymer, body tissue from another organism, body tissue from another individual, body tissue from another species, a metal, stainless steel, titanium, high molecular weight polyethylene, alloy, alloy of aluminum, and alloy of tinanium.

13. A neuromuscular stimulator electrode for use in vivo, comprising:
an electrically conductive material; and
one or more living muscle cells,

wherein the one or more living muscle cells forms a protective sheath enveloping at least most of the electrically conductive material.

14. The neuromuscular stimulator electrode of claim 13, wherein the one or more living muscle cells comprises a multinucleated fusion product of myoblasts.

15. The neuromuscular stimulator electrode of claim 13, wherein the protective sheath envelopes at least 85% of the electrically conductive material.

16. The neuromuscular stimulator electrode of claim 13, wherein the protective sheath envelopes at least 95% of the electrically conductive material.

17. The neuromuscular stimulator electrode of claim 13, wherein the muscle cells are obtained by culture from a human muscle biopsy.

18. The neuromuscular stimulator electrode of claim 13, wherein the electrically conductive material comprises a metal and the coated electrode is a Peterson-like electrode.

19. The neuromuscular stimulator electrode of claim 13, wherein the electrically conductive material comprises a substance selected from the group consisting of activated iridium, porous tantalum, an organic conductor, carbon and stainless steel.

20. The neuromuscular stimulator electrode of claim 19, wherein the organic conductor is carbon fiber prepared by * high temperature oxidation etc. add here..

21. The neuromuscular stimulator electrode of claim 13, further comprising a material for attachment to a muscle in an animal body.

22. The neuromuscular stimulator electrode of claim 21, wherein the material is selected from the group consisting of one or more hooks, one or more pins, one or more barbs, a glue, a molecular coating that binds non-specifically to muscle, a molecular coating that binds specifically to muscle, one or more mechanical bands,

one or more wires, one or more tapes, one or more Velcros, one or more molecular Velcros, and one or more sutures.

23. The neuromuscular stimulator electrode of claim 13, wherein the material is biodegradable during conditions of use in vivo.

24. The neuromuscular stimulator electrode of claim 13, wherein the electrically conductive material has a length that elongates during use.

25. The neuromuscular stimulator electrode of claim 14, wherein the electrically conductive material is selected from the group of a coiled spring, a coil, an elastic material, a thread, a fabric, a rubber and a multiply folded elongated conductor.

26. The neuromuscular stimulator electrode of claim 13 wherein the conductive material is primarily porous and comprises within its pores one or more nerve cells.

27. The neuromuscular stimulator electrode of claim 26, wherein the conductive material comprises a carbonized thread and/or fabric.

28. The neuromuscular stimulator electrode of claim 13, wherein the conductive material is porous and the neuromuscular stimulator electrode further comprises a substance selected from the group consisting of a water absorbing polymer, an extracellular scaffold protein, and a leachable substance that affects myoblast migratory behavior..

29. The neuromuscular stimulator electrode of claim 28, wherein the water absorbing polymer is selected from the group consisting of a synthetic polymer, a collagen, a starch, amylopectin, amylose, polyacrylamide, polyethylene glycol, Dacron, polyurethane, crosslinked collagen, phospholipid polymer, hydrogel and carbon fiber.

30. The neuromuscular stimulator electrode of claim 28, wherein the extracellular scaffold protein is selected from the group consisting of a laminin, laminin-1, fibronectin, a collagen, type I collagen, type II collage, type IV collagen,

thrombospondin-I, lecithin-oxytetracycline-collagen matrix, a galactin, galectin-1, vitronectin, and von Willebrand protein.

31. The neuromuscular stimulator electrode of claim 30, wherein the extracellular scaffold protein is fibronectin and wherein the fibronectin is macroscopically oriented in parallel strands.

32. The neuromuscular stimulator electrode of claim 28, wherein the leachable substance that affects myoblast migratory behavior is selected from the group consisting of platelet derived growth factor, culture media extract from culture of macrophages, and low molecular weight culture media extract from culture of damaged muscle.

33. The neuromuscular stimulator electrode of claim 31, wherein the leachable substance is bound to at least one or more water absorbing polymers, conductive organic polymer or extracellular scaffold protein with an association constant of less than 0.001.

34. A neuromuscular stimulator electrode for use in vivo, comprising:
an scaffold that provides mechanical strength for holding and allowing the transplant of one or more living neurons; and
one or more living muscle cells,
wherein the one or more living muscle cells forms a protective sheath enveloping at least part of the scaffold.

35. The neuromuscular stimulator electrode of claim 34, comprising one or more organic fibers.

36. The electrode of claim 34, wherein the scaffold further comprises an extracellular scaffold protein selected from the group consisting of a laminin, laminin-1, fibronectin, a collagen, type I collagen, type II collage, type IV collagen, thrombospondin-I, lecithin-oxytetracycline-collagen matrix, a galactin, galectin-1, vitronectin, and von Willebrand protein.

37. The electrode of claim 36, wherein the scaffold comprises fibronectin and the fibronectin is organized as parallel fibrils that form one or more sheets for support of the one or more neurons.

38. The electrode of claim 37, wherein the one or more sheets are formed by coating on a substrate.

39. A method of preparing a neuromuscular stimulatory electrode comprising:
incubating a conductive electrode material in the presence of myoblast cells to allow contact of the electrode material with multiple myoblast cells;
allowing or inducing the myoblasts in contact with the electrode material to fuse, thereby forming a continuous multinucleated form covering at least a substantial portion of the electrode material.

40. The method of claim 39, wherein the electrode is a Peterson-like electrode.

41. The method of claim 39, wherein the electrode is coated with at least one protein prior to contact with myoblast cells.

42. The method of claim 41, wherein the protein is selected from the group consisting of a laminin, laminin-1, fibronectin, a collagen, type I collagen, type II collage, type IV collagen, thrombospondin-I, lecithin-oxytetracycline-collagen matrix, a galactin, galectin-1, vitronectin, and von Willebrand protein.

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(57) Abstract: Bioactive electrodes for implantation into existing muscles are provided that enhance the functioning of the muscles. The electrodes may be coated with myogenic cells and may be transplanted as a living package, surrounded by a biologically degradable covering, into a weak muscle. Electrode surfaces may be coated with biologically active substances such as scaffold proteins that encourage migration and binding of myogenic cells and precursors to neurons. An electrode may slowly release trophic factors and may pre-train associated myogenic cells and/or nerve cell precursors by repeated electrical stimulation of the electrode in vitro in the presence of the cells. By transplanting myogenic cells along with the electrode, a new and powerful tool for muscle boosting, repair or augmentation is provided.

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STN: Medline CAPlus EMBASE Biosis US Patents Derwent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2001/0055590 A1 (KURACHI) 27 DECEMBER 2001 (27.12.2001); see entire document.	1-42
A	US 6,284,242 B1 (KURACHI) 04 September 2001 (04.09.2001); see entire document.	1-42
A	US 6,444,642 B1 (SKLAR, et al.) 03 September 2001 (03.09.2001); see entire document.	1-42
A	US 6,087,324 (IGARI, et al.) 11 July 2000 (11.07.2000); see entire document.	1-42

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